Research News

Reinforcing the idea of signalling in the stomatal pathway

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Stomata are plant epidermal structures that regulate gas exchange with the atmosphere. In *Arabidopsis*, mutations in *TOO MANY MOUTHS* (*TMM*) disrupt a range of processes related to stomatal development and patterning. Recently, the sequence and the expression pattern of *TMM* were reported. *TMM* encodes a leucine-rich-repeat-containing, receptor-like protein that lacks a cytoplasmic kinase domain and that is expressed in postprotodermal cells. Several lines of evidence suggest that TMM and STOMATAL DENSITY AND DISTRIBUTION1, a putative subtilisin-like serine protease, might act in the same signalling pathway.

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Stomata are plant epidermal structures that control essential functions such as the uptake of CO_2 for photosynthesis and the loss of water vapour during transpiration. They consist of two guard cells (GCs) that delimit a pore (Fig. 1). Changes in the shape of the GCs, in response to turgor pressure, control the pore opening and closure, and hence gas exchange between the plant and the atmosphere. Gas exchange also depends on the spacing of stomata. As a general rule for all plant species, stomata never develop next to one another, but instead they are separated by a number of non-stomatal cells. A recent study has shed light on how the spacing of stomata is regulated in *Arabidopsis* [1].

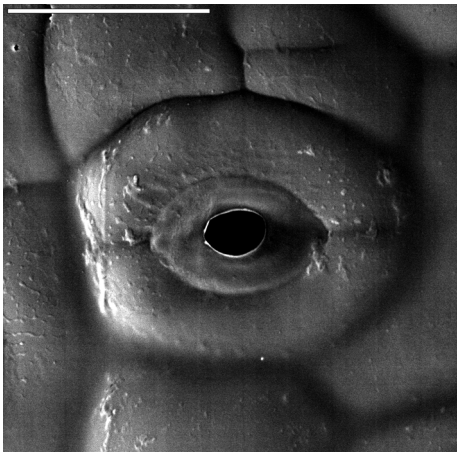

Fig. 1. CRIO-SEM of a stoma in *Arabidopsis*. Two guard cells delimit the stomatal aperture. Controlling the size of stomatal pore regulates gas exchange between the plant and the atmosphere. Scale bar: 10 μm.

In *Arabidopsis* leaf and cotyledon, stomatal development starts with an unequal cell division of a protodermal cell called a meristemoid mother cell (MMC) [2] (Fig. 2a). This cell division produces a small and triangle-shaped meristemoid and a neighbouring cell. These meristemoids are self-renewing cells, giving rise to new meristemoids. However, after a number of unequal cell divisions (from zero to three divisions), meristemoids lose their stem cell fate to become a rounded cell called a guard mother cell (GMC). The GMC undergoes a final, equal cell division that gives rise to the two GCs that form the stoma. These cell divisions in the stomatal pathway are precisely orientated in a spiral pattern that tends to place the stoma in the centre of the resulting multicellular complex (Fig. 2a).

The acquisition of MMC identity is prevented in cells that contact two stomata or meristemoids (Fig. 2b, top). However, many other epidermal cells can assume an MMC identity [2]. When MMC identity is assumed by a cell that makes contact with a stoma (or meristemoid), the MMC division is orientated so that the new meristemoid forms away from the existing stoma [2,3] (Fig. 2b, bottom). This is the main mechanism preventing the formation of adjacent stomata in wild-type plants [2] (Fig. 3a).

The role of *TOO MANY MOUTHS*

The recessive mutation *tmm* triggers a complex phenotype affecting the initiation of stomatal pathway, and the number and orientation of cell divisions that the MMC undergoes. The *tmm* mutation both promotes and suppresses stomata formation depending on the organ (or region). It eliminates stomata formation in the stem [3,4], hypocotyl and on the adaxial side of the sepal [4], but it increases stomatal initiation in the cotyledons [3,4], anthers and on the sepal abaxial side [4]. It reduces stomatal initiation and even produces stomatal gradients in other organs [4].

In the leaf and cotyledon, the *tmm* mutation allows the acquisition of MMC identity in cells that make contact with

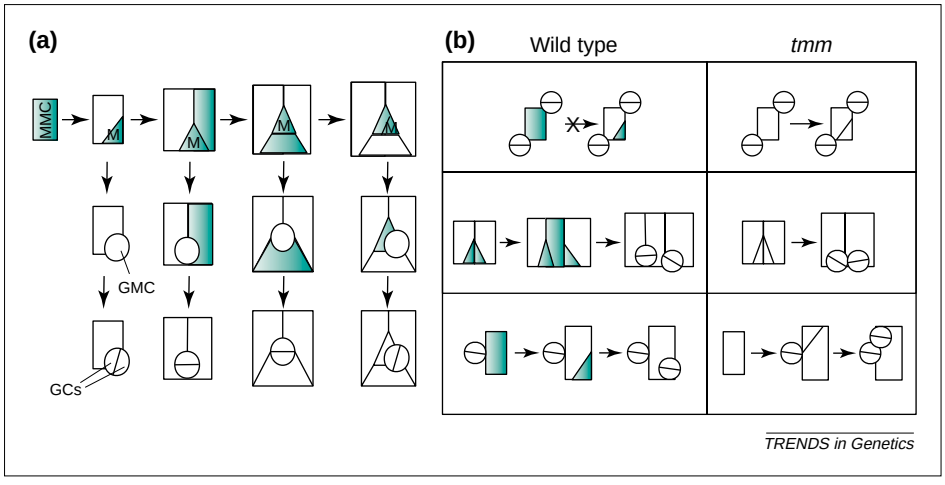

Fig. 2. Stomatal development and *TMM* expression pattern in an *Arabidopsis* leaf. (a) Stomatal lineages in wild-type plants begin with an unequal division from a meristemoid mother cell (MMC). It produces a small and triangle-shaped meristemoid (M) and a neighbouring cell. The number of subsequent unequal divisions that undergoes the meristemoids, before the formation of the rounded guard mother cell (GMC), varies from zero to three. The GMC undergoes an equal division producing the two guard cells (GC) that form the stoma. In a *tmm* mutant, the number of MMCs that produce stomata after the first or second unequal cell division is increased. (b) TMM also regulates other processes during stomatal formation. It prevents that cells in contact with two stomata (or meristemoids) initiate stomatal lineages (top). TMM corrects pattern 'mistakes' producing intervening cells between adjacent meristemoids, preventing the development of stomatal clusters; the formation of these adjacent meristemoids is a rare event (middle). TMM regulates the orientation of the cell division plane of MMCs in contact with a stoma (or meristemoid), placing the meristemoids away from the existing stomata (bottom). This is the main mechanism that avoids the development of stomatal clusters. In (a) and (b), green indicates *TMM* expression.

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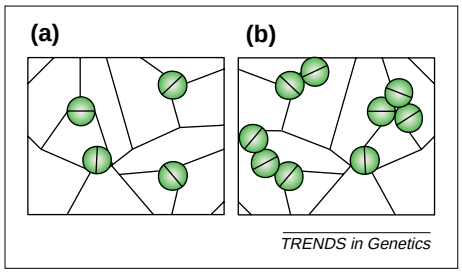

Fig. 3. Stomatal pattern in the leaf of wild-type and *tmm* plants. (a) In wild-type plants, stomata are surrounded by a stomata-free region. (b) This is disturbed in *tmm* mutant, where stomata develop together.

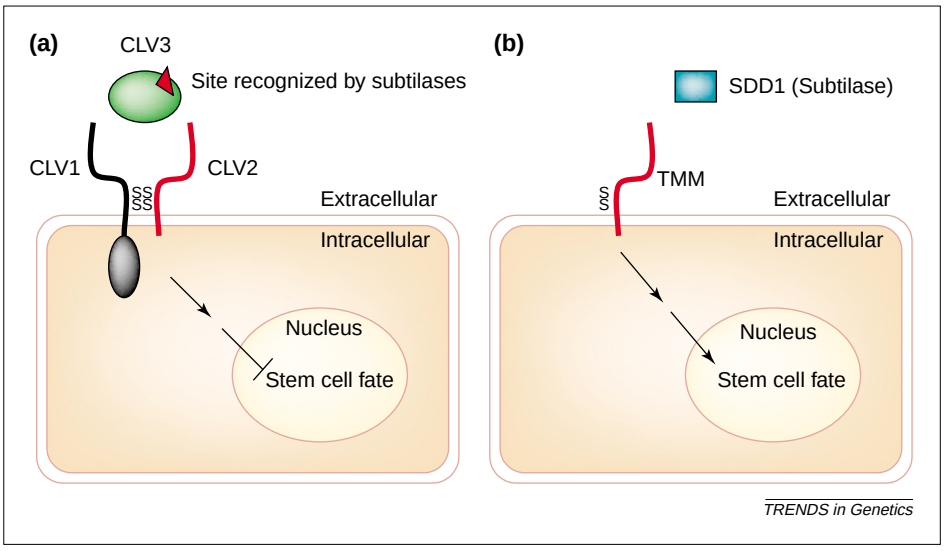

Fig. 4. Meristems and meristemoids: similar molecules and opposite roles. (a) In the shoot meristem, CLV3 is the ligand for the CLV1/CLV2 complex. Ligand binding triggers a signal transduction pathway that results in the repression of stem-cell fate maintenance. (b) TMM, a leucine-rich-repeat-containing, receptor-like protein similar to CLV2, is required for stem cell fate maintenance of the meristemoids. *SDD1* encodes a subtilisin-like serine protease, and it is also required to maintain the stem fate of the meristemoids. Both the absence of a cytoplasmic kinase domain in TMM and the presence of paired cysteines suggest that it might form disulfide bridges with a partner. In addition, the presence of a putative dibasic processing site recognized by subtilases in CLV3, suggests that a CLV3 homolog might be processed by SDD1. TMM and SDD1 also reduce the number of neighbouring cells entering the stomatal pathway, and they regulate the orientation of cell division planes in these cells, thus placing meristemoids away from stomata or meristemoids

two stomata or meristemoids [2] (Fig. 2b, top). In wild-type plants, occasionally two meristemoids make direct contact (a 'pattern mistake'). When this happens, one meristemoid produces an intervening cell that separates it from the neighbouring meristemoid, avoiding the formation of stomatal clusters [2]. However, the *tmm* mutation suppresses the ability to correct pattern mistakes, and produces a small number of adjacent stomata [2] (Fig. 2b, middle).

The number of MMCs that form early stomata (after the first or second unequal cell division) increases in the leaf and cotyledon of the *tmm* mutant [2] (Fig. 2a). The orientation of the cell division plane of MMCs in contact with stomata (or meristemoids) is also disturbed [2,3] (Fig. 2b, bottom); in the *tmm* mutant, many MMCs divide so that the new meristemoids contact the existing stomata (or meristemoids), forming a large number of stomatal clusters [2,3] (Fig. 3b).

The *tmm* phenotype indicates that TMM has multiple roles: (1) organ- and region-dependent regulation of stomatal pathway initiation; (2) repression of stomatal pathway initiation in cells making contact with two stomata or meristemoids; (3) correction of pattern mistakes; (4) maintenance of stem cell fate in meristemoids; and (5) proper orientation of cell division of MMCs that contact stomata. These TMM functions suggest cell signalling in the stomatal pathway [5].

The *TOO MANY MOUTHS* sequence and expression pattern

TMM encodes a leucine-rich-repeat (LRR)-containing, receptor-like protein (RLP), carrying an extracellular domain composed of ten uninterrupted LRRs and a presumptive transmembrane domain [1]. LRR motifs often participate in protein–protein interactions [6], suggesting that TMM has a role in a signalling pathway through binding of an extracellular protein or peptide ligand.

The TMM structure is very similar to CLAVATA2 (CLV2), another LRR-RLP that represses the proliferation of undifferentiated cells at the shoot meristem [7,8]. Similarly to CLV2 [8], TMM contains paired cysteines in both the amino and carboxyl non-LRR regions, and it lacks a cytoplasmic kinase domain. Nadeau and Sack [1] infer from this that TMM might interact with a partner that provides the cytoplasmic domain to transduce information across the membrane, in the same way as CLV2 seems to interact with CLV1 [8].

TMM expression is restricted to shoot epidermal cells, excluding the apical meristem [1]. The expression is detected in young leaf primordia, and it moves from the apical to the basal end of the leaf as it matures. *TMM* expression is not detected in fully expanded leaves. A detailed analysis of the expression pattern in developing leaves shows that the gene is switched on in cells adjacent to two stomata or meristemoids (Fig. 2b, top). This supports the idea that TMM negatively regulates stomatal initiation in these cells, as suggested by the mutant *tmm* phenotype.

TMM is also expressed in many small cells, regardless of whether they are in contact with a stoma or meristemoid. The authors argue that this expression pattern does not mark a population of cells that will necessarily enter the stomatal pathway, because many *TMM*-expressing cells do not divide [1]. Instead, *TMM* expression marks those cells where the initiation of the stomatal pathway is possible. As the Nadeau and Sack state, this is supported by the fact that *TMM* expression is rare in larger epidermal cells or in older cells making contact with a stoma, GMC or meristemoid (Fig. 2a); these cells rarely enter the stomatal pathway. It is important to note that although *TMM* transcripts mark competence to form stomatal lineages, the initiation of the stomatal pathway is not dependent on TMM function, in agreement with the *tmm* phenotype. As Nadeau and Sack conclude [1], in cells adjacent to a stoma or meristemoid, *TMM* expression is consistent not only with stomatal lineage competence, but also with a role in the correct orientation of the cell division plane that places new meristemoids away from the pre-existing stomata (Fig. 2b, bottom).

TMM is not expressed in stomata but in Ms and, to a lesser extent, in GMC (Fig. 2a and 2b, middle). Consistent with the *tmm* phenotype, the authors infer that TMM has a role in the regulation of stem cell fate of the meristemoids and in the correction of pattern mistakes. It should be noted that

TMM might act either to extend self-renewal capacity of the meristemoids or to repress an early stomatal differentiation.

TOO MANY MOUTHS and STOMATAL DENSITY AND DISTRIBUTION1: do they act in the same pathway?

TMM and STOMATAL DENSITY AND DISTRIBUTION1 (SDD1) share a number of functions during stomata formation in the leaf [2,9], suggesting that they might act in the same pathway [10]. They both reduce the number of cells adjacent to stomata entering the stomatal pathway; they regulate the orientation of the cell division plane in cells making contact with stomata or meristemoids; and they control the balance between meristemoids renewal and stomatal differentiation. *SDD1* encodes a member of the subtilisin-like serine protease family, and it has been suggested that SDD1 might activate a proteinaceous signal molecule precursor or a precursor of a receptor [9].

But how might TMM and SDD1 interact? Because TMM lacks a cytoplasmic domain, it might need to dimerize with a protein that provides a cytoplasmic domain to transduce the signal from the cell surface to the cytoplasm. Receptor-like kinases have a kinase cytoplasmic domain and might interact with TMM, producing a heterodimeric receptor that should be activated by a ligand. We speculate that an SDD1-processed molecule might be recognized by the extracellular domain of the hypothetical heterodimer, triggering a transduction pathway that results in the execution of the functions described above. This model mirrors those in which the CLV3 ligand binds to and activates the CLV1/CLV2 receptor complex [11] (Fig. 4). Intriguingly, CLV3 contains a putative dibasic processing site recognized by subtilases [9]. It is important to note that, although the activation of the CLV1/CLV2 complex represses stem-cell maintenance at the shoot meristem [11], the presumptive TMM-mediated signalling promotes the opposite process in the leaf and cotyledon epidermis (Fig. 4).

The comparison between *tmm* and *sdd1* phenotypes suggests that TMM might interact with SDD1 regulating the phenotypes disrupted by both mutations. Such comparison also indicates that TMM acts independently of SDD1, regulating processes that are disrupted only by *tmm* mutation; for example, stomatal formation and pattern in hypocotyls or fruit. Assuming the model proposed above, in these organs TMM might be not activated by an SDD1-processed factor, but by a different ligand (or ligands).

Conclusions

The molecular characterization of TMM has reinforced the idea that cell signalling guides stomatal fate in *Arabidopsis*. Exciting challenges for the future include finding the signals that trigger the TMM receptor, which molecules relay its downstream effects and whether TMM needs to heterodimerize. The absence of a cytoplasmic domain in TMM strongly suggests that it interacts with a partner. The existence of candidate genes to be TMM partners [12] and the relative ease of generating knockouts might facilitate its identification. However, the large number of candidates proposed to dimerize with TMM might make this a difficult task. A combination of genetic, molecular and biochemical analysis will be required to identify this and other components of the stomatal development pathway. We have the tools to hand to answer the key questions of how a stomatal precursor knows where it is and who its neighbours are.

Acknowledgements

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